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Research Article

Separation and Purification of Polyphenols from Pericarpium Granati Using Macroporous Resins and Evaluation of its Anti-*Streptococcus mutans* Activity *in vitro*

[1,2,3]Xudong Jiang, [4]Weihao Yang, [4]Chuanjian Zhou, [4]Kailing Lu and [2,3]Cuiwu Lin

[1]School of Medicine, Guangxi University of Science and Technology, 545005 Liuzhou, China
[2]School of Chemistry and Chemical Engineering, Guangxi University, 530004 Nanning, China
[3]Key Laboratory of Applied Chemistry Technology and Resource Development, Guangxi Colleges and Universities, 530004 Nanning, China
[4]Liuzhou LMZ Co., Ltd., 545006 Liuzhou, China

Abstract

Background: The optimal parameters of separation and purification of polyphenols from Pericarpium Granati were determined and the antibacterial activities against *S. mutans in vitro* were investigated. **Methodology:** Through comparing the static adsorption/desorption properties of five kinds of tested macroporous resins for polyphenols, HPD-450 resin was selected to investigate optimal purification parameters by dynamic curve experiments. The optimal purification conditions as follows: 9.0 BV of feeding solution at concentration of 3.2 mg mL^{-1} through the column at 3.0 BV h^{-1}, then for desorption 60% ethanol-aqueous 3.5 BV at 2.0 BV h^{-1} eluted. The content of polyphenols was increased from 34.9±1.6% to 71.1±3.1% with a recovery yield of 85.5±3.7% according to the optimal parameters. **Results:** The present study showed that the MIC of purity of polyphenols was 6.25 mg mL^{-1}. The acidogenicity of *S. mutans* can be significantly inhibited when the concentration was up to or higher than 1.56 mg mL^{-1} and the adherence of *S. mutans* can be significantly inhibited when the concentration was up to or higher than 3.13 mg mL^{-1} as well. **Conclusion:** The results demonstrated that polyphenols from Pericarpium Granati have significant activity against *S. mutans* and it is a good candidate for further development as an oral care agent for preventing dental caries.

Key words: Pericarpium Granati, polyphenols, resins, *Streptococcus mutans*

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Corresponding Author: Cuiwu Lin, School of Chemistry and Chemical Engineering, Guangxi University, No.100 Daxue East Road, 530004 Nanning, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dental caries is a common microbial infectious disease, which involves the hard tissue of tooth. *Streptococcus mutans* is one of the dominating and resident germs of oral, also is the dominating germ factor of the occurrence of caries is discussed by Loesche[1]. *Streptococcus mutans* has the capacity of growth, adherence and acidogenicity at tooth surfaces and lead to further demineralization during the formative process of dental plaque[2]. Therefore, there has been increasing attention focused on inhibitory effect of *S. mutans* to discover new compounds. The use of natural products and their preparations to treat diseases has been extensively studied based on their biological activities for various purposes worldwide have received increased attention in recent years following the described[3-5]. The researchers have discovered some natural plants such as *Mangnolia officinalis*, *Galla chinesis* and *Bauhinia championii* (Benth.) Benth, etc., rich in polyphenols have exhibited significant anticaries activity as discussed[6,7].

Pomegranate contains substantial amounts of polyphenol compounds such as ellagic acid, ellagic tannins and gallic acid and the highest content is in Pericarpium Granati as discussed by Li *et al.*[8]. The separation and purification of polyphenols from Pericarpium Granati technology used SP-700, D-141 and HZ-818 resins as described by Zhang *et al.*[9], Zhu *et al.*[10] and Tang *et al.*[11]. However, SP-700, D-141 and HZ-818 resins were expensive and not easy to purchase. Moreover, previous studies showed that polyphenols from Pericarpium Granati have a variety of biological activity effects such as anti-tumor, antioxidant, bactericidal, anti-inflammatory[12-14]. However, it has not been reported the effects on *S. mutans*. In this study, a crude extract was prepared from Pericarpium Granati, the appropriate resin was selected for separation and purification of polyphenols by static adsorption/desorption experiment. The optimal adsorption/desorption parameters were determined by dynamic curve. The objective was to develop more practical technology for providing high-purity polyphenols from Pericarpium Granati crude extract using macroporous resins, further to evaluate the anticariogenic activities against *S. mutans in vitro*, which is provided scientific evidence for further development and enhanced the application of polyphenols from Pericarpium Granati on caries prevention.

MATERIALS AND METHODS

Materials and reagents: The sample of Pericarpium Granati was purchased from Liuzhou, China, identified by Professor Guangwei Huang (Liuzhou LMZ Co., Ltd., China). Gallic acid standard (98%) (Kuer Chem. Co., Ltd., China). *Streptococcus mutans* strain (ATCC 25175) (GIMCC, China). The TPY liquid medium (Guangdong HKM Co., Ltd., China).

Total phenolic content: The total phenolic content was determined using the Folin-Ciocalteu assay described by Dorman *et al.*[15] with slight modification. The 2.0 mL of sample solution was mixed with 4.0 mL of Folin-Ciocalteu reagent and 10.0 mL of 20% sodium carbonate solution and then diluted to 50 mL with pure water. The absorbance at 760 nm was measured using a UV-visible spectrophotometer (UV-2000, Shanghai Analytical Instrument Overall Factory, Shanghai, China) after 2 h of incubation at 30°C. Gallic acid was used to generate a calibration curve at concentrations of 20-120 μg mL^{-1}, the linear regression equation was $y = 0.0067x+0.0102$ and $R^2 = 0.9987$.

Preparation of crude extract from Pericarpium Granati: The powder of Pericarpium Granati (40 g) was extracted with 800 mL 60% ethanol-aqueous solution at 65°C for 100 min as discussed by Jiang *et al.*[16]. The filtrates were collected and concentrated to a crude extract with a rotary evaporator at 65°C.

Adsorbents: Five kinds of tested macroporous resins including D101, AB-8, HPD-100, HPD-450 and HPD-600 were cheap and easy to obtain commercially, purchased from Donghong Chemical Co., Ltd. (Shandong, China) and their unique physical properties are listed in Table 1.

The pretreatment of macroporous resins following the described by Wang *et al.*[17] with slight modification. The resins were soaked in 3 Bed Volumes (BV) of 95% ethanol-aqueous overnight, washed successively with ethanol and pure water until the eluent was clear. In succession, soaked in same amount 1 M NaOH and HCl for 6 h, washed with pure water until the eluent was neutral[18]. Then the resins were dried at 65°C for 24 h, subsequently placed in a desiccator for later use.

Static adsorption, desorption and kinetics experiments: For the purpose to investigate the adsorption/desorption properties of five kinds of tested resins for polyphenols, the static adsorption/desorption and theirs kinetics experiments were performed as follows[19]: Dry resins 2.0 g and 20 mL of sample solution at concentration of 10.0 mg mL^{-1} were put into a 50 mL glass-stoppered conical flask and then flask was consecutively shaken for 8 h with 120 rpm at 25°C, the static

Table 1: Physical properties of the tested resins

Resin types	Polarity	Surface area ($m^2 g^{-1}$)	Average pore diameter (nm)	Particle diameter (mm)
D101	Non-polar	500-550	9.0-10.0	0.3-1.25
AB-8	Weak-polar	450-500	12.0-16.0	0.3-1.20
HPD-100	Non-polar	650-700	8.5-9.00	0.3-1.25
HPD-450	Weak-polar	500-550	9.0-11.0	0.3-1.20
HPD-600	Polar	550-600	8.0	0.3-1.20

adsorption capacity and adsorption ratio were determined at adsorption equilibrium. During the adsorption process, the adsorption kinetics was studied by monitoring the content of polyphenols at equal time intervals. After completion of the adsorption process, filtered and washed the resins with pure water and then the separated resins were put into another 50 mL glass-stoppered conical flask. Subsequently, 20 mL of 60% ethanol-aqueous solution was added and the flask was consecutively shaken for 8 h with 120 rpm at 25°C, the static desorption capacity and desorption ratio were determined at desorption equilibrium. During the desorption process, desorption kinetics was studied by measuring the concentration of polyphenols at equal time intervals in the desorption solution. The optimal resin was selected on the basis of theirs adsorption/desorption properties which were calculated in term of the following equations.

Adsorption capacity (mg g^{-1}):

$$Q = \frac{C_0 - C_1}{W_1} \times V_1 \quad (1)$$

Adsorption ratio (%):

$$A = \frac{C_0 - C_1}{C_0} \times 100\% \quad (2)$$

Desorption ratio (%):

$$D = \frac{C_2 \times V_2}{(C_0 - C_1) V_1} \times 100\% \quad (3)$$

where, C_0, C_1 and C_2 are the primary and various adsorption/desorption stage concentrations (μg mL^{-1}) of polyphenols, respectively, V_1 and V_2 are the volume of the sample and ethanol-aqueous solution (mL), respectively and W_1 is the weight of dried resin (g).

Dynamic adsorption/desorption experiments

Optimization of the feeding parameter: A series of feeding parameters, including concentration, flow rate and volume were optimized. The general procedure, tested resins were wet-packed with 20 mL (a bed volume, 1 BV). After feeding, the eluent was collected and the concentration of polyphenols or adsorption ratio was determined according to the calibration curve[20,21]. The sample solutions at concentration 2.0, 2.4, 2.8, 3.2, 3.6, 4.0 mg mL^{-1} flowed slowly through the column at rate of 3.0 BV h^{-1}, respectively. The eluent was collected and the optimal feeding concentration was determined according to the dynamic adsorption ratio curve. On the other hand, the feeding flow rate experiment as follows. The sample solution at concentration of 3.2 mg mL^{-1} flowed through the column with the rate of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 BV h^{-1}, respectively. The optimal feeding flow rate was determined according to the dynamic adsorption ratio curve. At last, the sample solutions at concentration of 3.2 mg mL^{-1} flowed through the column at 3.0 BV h^{-1}. The eluent was collected every 20 mL (1 BV) and the optimal feeding volume was determined according to the leakage point.

Optimization of the eluent parameter: A series of eluent parameters, including concentration, flow rate and volume were optimized. The general procedure, test resins were wet-packed with 20 mL (a bed volume, 1 BV), the adsorption-saturation was reached according to the adsorption parameters obtained, eluted the column with solution. The elution effluent was collected and the concentration of polyphenols or desorption ratio was determined according to the calibration curve. The optimal eluent concentration was investigated using a series of ethanol-aqueous solutions (0, 20, 40, 60, 80 and 100% (v/v), respectively) eluted the column at 3.0 BV h^{-1}. The optimal eluent concentration was determined according to the dynamic desorption curve. The optimal eluent flow rate was evaluated by comparing the 60% ethanol-aqueous solution and the rate was set at 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 BV h^{-1}, respectively. The optimal eluent flow rate was determined according to the dynamic desorption curve. The optimal desorption eluent volume was investigated using 60% ethanol-aqueous solution eluted the column at rate of 2.0 BV h^{-1}, every 10 mL (0.5 BV) of the eluent was collected, the optimal eluent volume was determined according to the dynamic concentration curve as discussed by Liu *et al.*[22].

Table 2: Static adsorption/desorption properties of the tested resins

Macroporous resin	Adsorption capacity (mg g^{-1})	Adsorption ratio (%)	Desorption ratio (%)
D101	23.6±1.1	67.4±3.1	85.2±3.9
AB-8	19.4±0.9	55.4±2.6	79.4±3.8
HPD-100	26.3±1.0	75.1±2.9	86.1±4.1
HPD-450	26.6±0.9	76.0±2.6	85.5±3.9
HPD-600	22.3±1.1	63.5±3.1	67.3±3.3

Anti-*Streptococcus mutans* activity experiments

Effect on growth of *Streptococcus mutans*: Minimal Inhibitory Concentration (MIC) was investigated by agar disc diffusion method as described by Khurram *et al.*[23]. A series of solutions at concentration from 50.0-1.56 mg mL^{-1} were done in TPY liquid medium, *S. mutans* (0.9×10^8 CFU mL^{-1}, 0.1 mL) were put into each culture tube containing previous TPY liquid medium (2.0 mL), anaerobic incubation for 48 h at 37°C. The MIC value according to no visible *S. mutans* produced growth with visual inspection.

Effect on adherence of *Streptococcus mutans*: According to the MIC value, glass surface adherence assay was investigated at concentrations of MIC and below according to methods previously described[24,25] with slight modifications, respectively. The *S. mutans* (0.9×10^8 CFU mL^{-1}, 0.1 mL) were pipetted into a glass burette by angle of 30°, which contained 10 mL of TPY liquid medium (different concentrations tested polyphenols and 1% sucrose), anaerobic incubation for 48 h at 37°C, supernatants were removed and washed three times with pure water. The adhered bacteria were resuspended into 5% NaOH solution and then the supernatant was removed by centrifuging, suspended in normal sodium and measured the OD value at 540 nm.

Effect on acidogenicity of *Streptococcus mutans*: A pH drop program was employed to evaluate the effect on acidogenicity of *S. mutans* as described by Joycharat[26]. *Streptococcus mutans* (0.9×10^8 CFU mL^{-1}, 0.1 mL) was grown in 10 mL TPY liquid medium which containing 1% sucrose and tested polyphenols at concentrations of MIC and below, respectively. The initial pH was adjusted to 7.4 and anaerobic incubation for 48 h at 37°C, measured terminal pH of supernatants and calculated pH reduction.

Statistical analysis: All of the experiments were performed in triplicate and the results were expressed as Mean±SD. Analysis of variance was performed by ANOVA test and a statistically significant ($p<0.05$) or very significant ($p<0.01$) was considered.

RESULTS AND DISCUSSION

Static adsorption/desorption experiments

Static adsorption/desorption properties of resins: The different properties of five kinds of tested resins for polyphenols were obtained as shown in Table 2. These resins can adsorb polyphenols through physical adsorption and the results were shown the adsorption capacity and adsorption ratio for polyphenols that HPD-100 and HPD-450 are higher than others at adsorption equilibrium because they have larger surface area or suitable polarity, which may provide the higher adsorption capacities for polyphenols. The slightly low adsorption capacity of AB-8 resin may be explained by its smaller surface area than that of D101 and HPD-100.

On the other hand, the desorption ratio were slightly higher than the adsorption ratio for all the resins. Polarity has played an important role for desorption capacity, generally a higher polarity imply a weaker capacity of desorption. Therefore, desorption capacity of HPD-600 was significantly lower compared with HPD-100, which had the highest desorption capacity. However, HPD-450 exhibited the second highest desorption capacity, may be due to the fact that HPD-450 have larger surface area than that of AB-8.

Static adsorption kinetic curve: The adsorption kinetic curve of polyphenols on five kinds of tested resins was screened. The results were shown in Fig. 1, the adsorption ratio was fast increases and then it gradually increased and finally slowed down until adsorption equilibrium. For HPD-450 resin, the adsorption capacity increases rapidly within 1 h, then almost reached adsorption equilibrium at 3 h, but HPD-100 resin reached adsorption equilibrium at 5 h. The reason is HPD-450 resin has suitable polarity to attract polyphenols and faster rate to reach adsorption equilibrium than HPD-100. However, the adsorption for HPD-600 resin reached to equilibrium at 3 h too, but the adsorption capacity far below HPD-450.

Static desorption kinetic curve: The effects of desorption time on the desorption rate of five kinds of tested resins were shown in Fig. 2. The kinetic desorption properties for polyphenols showed a similar tendency that desorption ratio of polyphenols increases accordingly. At the initial 1 h, the desorption process of all resins increased rapidly, however, the rate of adsorption and diffusion get to equal with the concentration increases, desorption ratio did not change obviously after 2 h, showed the desorption equilibrium almost achieved.

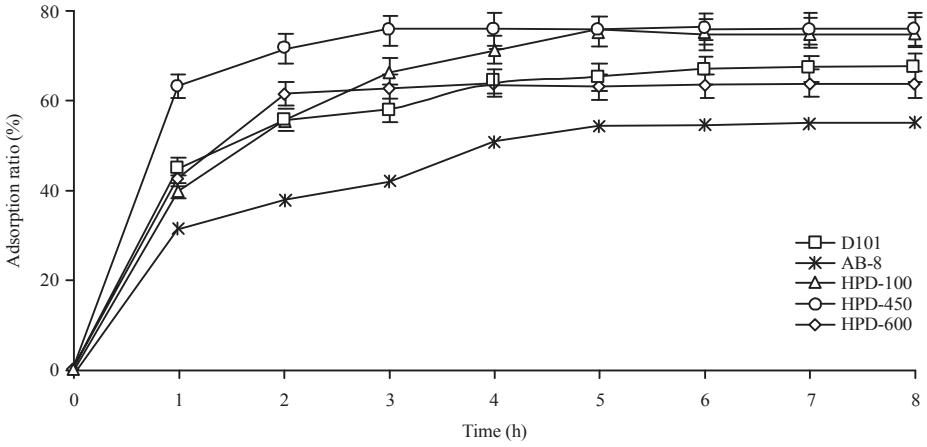

Fig. 1: Static adsorption kinetic curve of the tested resins

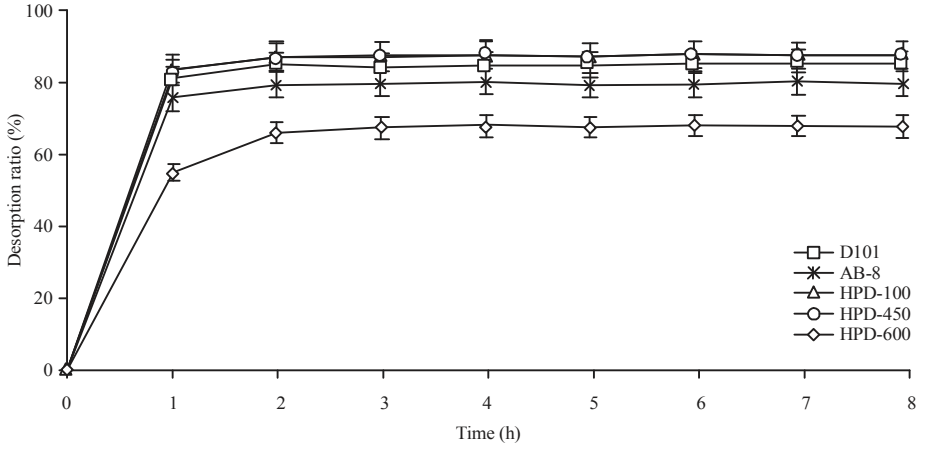

Fig. 2: Static desorption kinetic curve of the tested resins

Dynamic adsorption/desorption experiments: The HPD-100 and HPD-450 exhibited better adsorption/desorption capacity than those of others by static experiments. Although, HPD-450 resin had slightly lower desorption capacity than that of HPD-100, its adsorption ratio was slightly higher and has a shorter time to reach adsorption equilibrium compared with HPD-100. Therefore, HPD-450 resin was selected for further dynamic investigation with comprehensive consideration of adsorption/desorption properties.

Dynamic adsorption curve of feeding concentration: The process of adsorption includes adsorption and diffusion equilibrium between polyphenols and the resin. The adsorption reaches the breakthrough point when polyphenols starts to leak from the resin. Figure 3 shows the dynamic effects of different feeding concentration on adsorption. The adsorption ratio rapidly increased when the sample solution concentration from 2.0-3.2 mg mL^{-1} where is the highest adsorption ratio. After that, the adsorption ratio decrease gradually with the sample solution concentration increased. A high concentration of polyphenols causes to insufficient exchanged between polyphenols and resin and harm to the adsorption capacity. Therefore, the feeding concentration at 3.2 mg mL^{-1} was finally selected for further experiments.

Dynamic adsorption curve of feeding flow rate: Figure 4 shows as the adsorption ratio rapidly decreased as the feeding flow rate from 3.0-6.0 BV h^{-1}. It may be due to incomplete adsorption of polyphenols, since the flow rate of sample solution was faster. However, in the flow rate interval of 1.0-3.0 BV h^{-1}, in regard to the adsorption ratio, there was no significant difference at flow rate. It means the rate of change has little effect on the absorption ratio at low rate. Considering that a lower flow rate in general to affect efficiency, 3.0 BV h^{-1} was selected to further experiment as an optimal feeding flow rate.

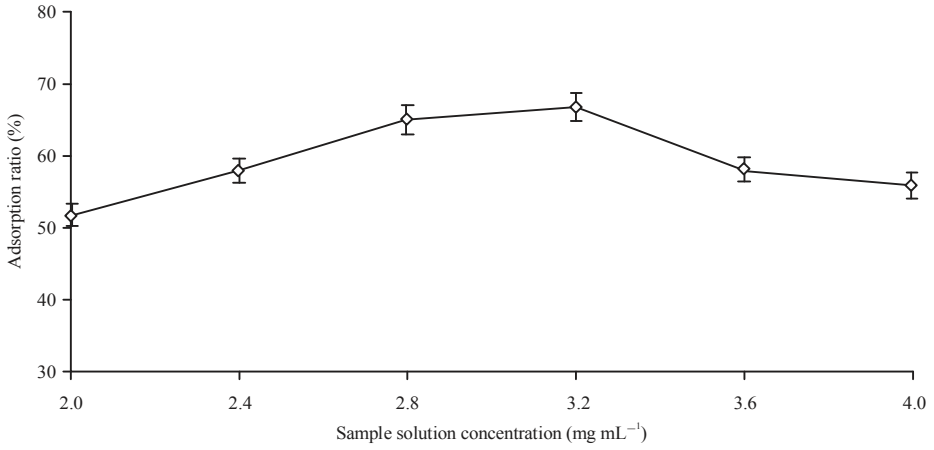

Fig. 3: Dynamic adsorption curve of feeding concentration

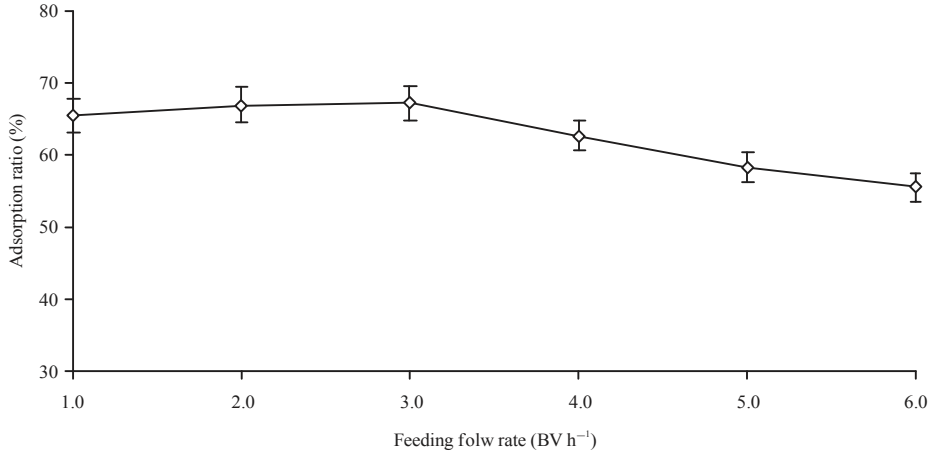

Fig. 4: Dynamic adsorption curve of feeding flow rate

Dynamic adsorption curve of feeding volume: The leakage point generally refers to the concentration of eluent reaches 5.0% of that of initial concentration. The leakage point curve experiments at the feeding flow rate of 3.0 BV h^{-1} is shown in Fig. 5. In the initial process, polyphenols in the sample solutions was almost completely adsorbed over the first 6.0 BV and the concentration of polyphenols closed to zero. However, the leakage process started gradually increased as the elution volume increased from 6.0-8.0 BV. The leakage process increased rapidly after volume of more than 9.0 BV and the maximum loading sample volume of the resin column was 9.0 BV, which the concentration of polyphenols in the effluent solutions reached the leakage point. At this stage, the adsorption capacity of the resin approaches saturation, it can be considered that there would no more accommodate polyphenols.

Dynamic desorption curve of ethanol-aqueous concentration: The dynamic desorption curve is used to display the profile of desorption process and obtained in terms of the concentration and the flow rate of eluent solution. Furthermore, eluent solution volume were used to perform desorption ratio experiments as well. Figure 6 shows, few desorption at the 0 and 20% at ethanol-aqueous concentration. However, the desorption ratio increased sharply, while the ethanol-aqueous concentration from 20-40%. Finally, the desorption ratio reached a peak at the 60%. After that, desorption ratio decrease gradually with the ethanol-aqueous concentration increased. Polyphenols from Pericarpium Granati exhibited certain polarity, the desorption ratio was highest while the ethanol-aqueous concentration closes to similar polarity. Therefore, 60% ethanol-aqueous

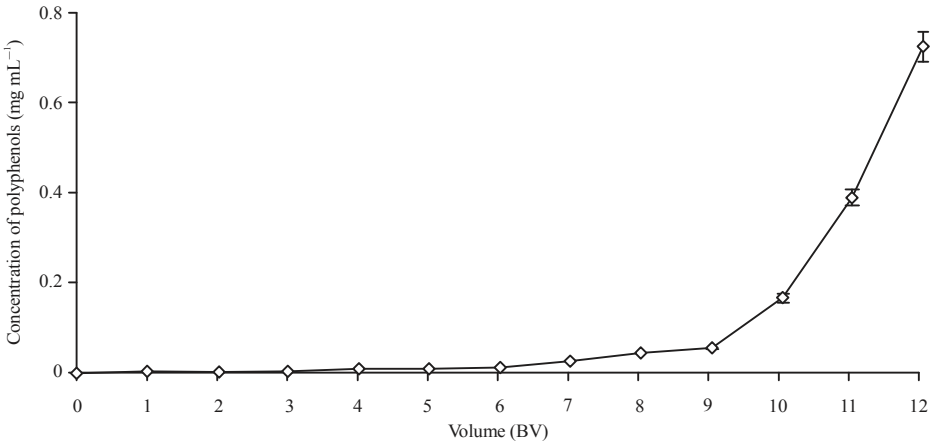

Fig. 5: Curve of adsorption leak of polyphenols

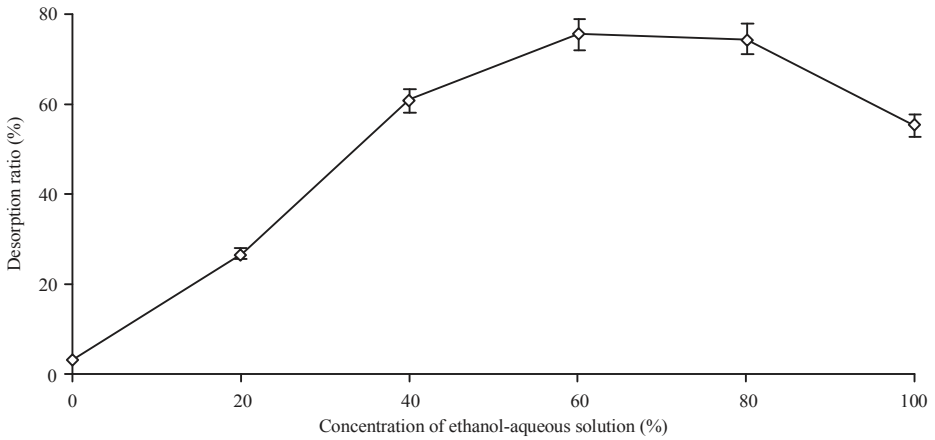

Fig. 6: Dynamic desorption curve of ethanol-aqueous concentration

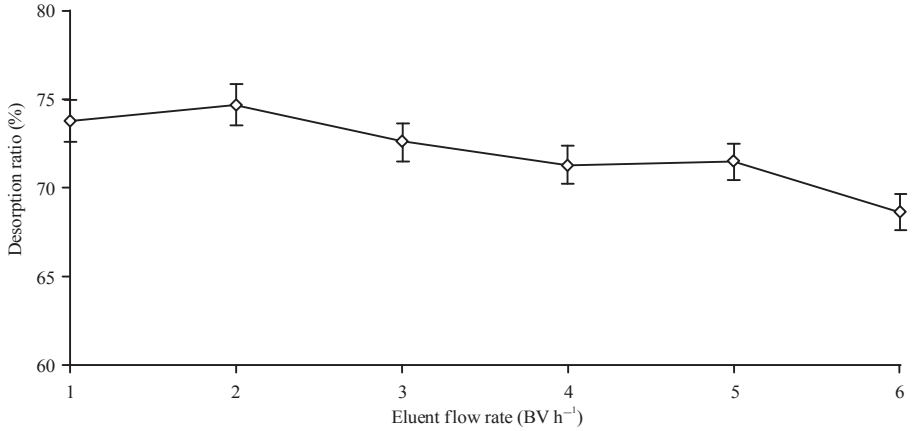

Fig. 7: Dynamic desorption curve of eluent flow rate

displayed the highest desorption ratio compared with that of others and as the optimal concentration to through the column.

Dynamic desorption curve of eluent flow rate: Figure 7 shows, in the flow rate interval of 1.0-2.0 BV h^{-1}, in regard to the desorption ratio, there was no significant difference at

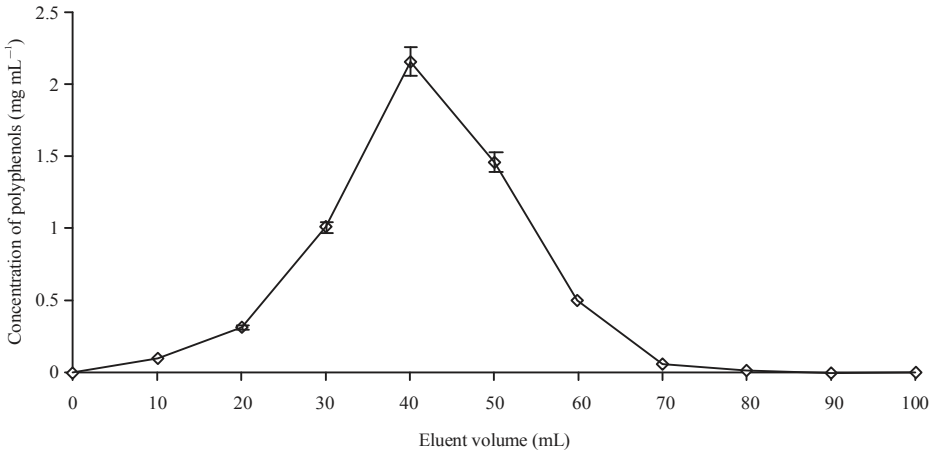

Fig. 8: Dynamic desorption curve of eluent volume

Table 3: Effect on growth of *Streptococcus mutans*

Concentration (mg mL^{-1})	Crude extract	Purification of polyphenols	Control group
25.0	-	-	+
12.5	-	-	+
6.25	+	-	+
3.13	+	+	+
1.56	+	+	+
0.078	+	+	+

+: Bacterial growth and -: No bacterial growth

Table 4: Effect on adherence of *Streptococcus mutans*

Concentration (mg mL^{-1})	OD_{540} values	p-values (compared with the control group)
6.25	0.380±0.014	p<0.01
3.13	0.478±0.023	p<0.01
1.56	0.651±0.028	p>0.05
0.78	0.675±0.030	p>0.05
0.39	0.687±0.034	p>0.05
Control group	0.693±0.029	----

Table 5: Effect on acidogenicity of *Streptococcus mutans*

Concentration (mg mL^{-1})	ΔpH values	p-values (compared with the control group)
6.25	0.875±0.058	p<0.01
3.13	3.050±0.106	p<0.01
1.56	3.520±0.092	p<0.05
0.78	3.637±0.135	p>0.05
0.39	3.860±0.108	p>0.05
Control group	3.883±0.081	----

flow rate. However, there have a slowly decreased of desorption ratio during the eluent flow rate from 2.0-6.0 BV h^{-1}. High flow rate reduced the time of adsorption-desorption exchange between the eluent and HPD-450 resin is harmful to desorption for polyphenols from resin. Take into account that lower eluent flow rate in general to affect efficiency, the eluent flow rate of 2.0 BV h^{-1} was selected for further experiments.

Dynamic desorption curve of eluent volume: The effects of eluent volume on desorption ratio were investigated with the aim of decreasing the solution consumption and making more efficient. The results were shown in Fig. 8. Polyphenols concentration in eluent solutions was increased rapidly with the eluent volume increased and reached a peak at the 40 mL (2.0 BV). After that, polyphenols concentration decrease gradually with the eluent volume increased. The concentration of polyphenols in the eluent closes to zero when the volume of elution was more than 70 mL (3.5 BV). It can be considered that there was no more polyphenols desorped by eluent solutions. Therefore, an eluent volume of 3.5 BV was selected as optimal parameter.

Purification of polyphenols on HPD-450 resin: The optimal parameters were as follows based on the dynamic experiments: The 9.0 BV of feeding solution at concentration of 3.2 mg mL^{-1} through the column at 3.0 BV h^{-1}, then for desorption 3.5 BV 60% ethanol-aqueous at 2.0 BV h^{-1} eluted. According to the optimal parameters, the content of polyphenols was increased from 34.9±1.6% to 71.1±3.1%, with a recovery yield of 85.5±3.7%, the results was consistent with the described by Zhang *et al.*[9], Zhu *et al.*[10] and Tang *et al.*[11]. However, HPD-450 resin has the advantage of cheap and easy to obtain commercially compared with SP-700, D-141 and HZ-818 resins that it worth to further study its industry applications.

Antibacterial activity against *Streptococcus mutans*: The antibacterial activities against *S. mutans* were evaluated using disc diffusion method and effects on growth, adherence and acidogenicity were investigated. The results of the antibacterial activity are given in Table 3-5.

Effect on growth of *Streptococcus mutans*: Table 3 shows, the MIC value of crude extract is 12.5 mg mL^{-1}, but the MIC value of purification of polyphenols is 6.25 mg mL^{-1} compared with 3.13-100.00 mg mL^{-1} as previously described by Jin *et al*.[6] Jiang *et al*.[7], the antibacterial activity on growth of *S. mutans* is outstanding. The purification of polyphenols has higher polyphenols content displayed higher inhibition ability for growth of *S. mutans*. The results illustrate polyphenols from Pericarpium Granati is one of the anticaries material basis therefore worthy of further study, which is consistent with previous research by Jin *et al*.[6] and Jiang *et al*.[7].

Effect on adherence of *Streptococcus mutans*: Table 4 shows that the effect on adherence of *S. mutans.* The results indicated that there were very significant effect on adherence of *S. mutans* when the concentration is greater than or equal to 3.13 mg mL^{-1} ($p<0.01$) compared with 0.78-12.5 mg mL^{-1} according to the described by Jin *et al*.[6] Jiang *et al*.[7] and He *et al*.[27], the inhibition ability is moderate. The *S. mutans* adhesion to the tooth surface, resulting in the formation of dental plaque, it is an important part of the formative of dental caries. Since the polyphenols from Pericarpium Granati has significant inhibitory effect on the adhesive function of *S. mutans* that it might be used in the area of dental caries prevention and treatment in the future.

Effect on acidogenicity of *Streptococcus mutans*: The effect on acidogenicity of *S. mutans* was given in Table 5. The authors found that polyphenols from Pericarpium Granati exhibited obviously inhibitory effects compared with control group when the concentration of purification of polyphenols is 1.56 mg mL^{-1} ($p<0.05$). There are very significantly inhibitory effects showed while the concentration of polyphenols is greater than 1.56 mg mL^{-1} ($p<0.01$). The results also indicated polyphenols from Pericarpium Granati have excellent effects of inhibition acidogenicity of *S. mutans* compared with 0.78-12.5 mg mL^{-1} following the described by Jin *et al*.[6] Jiang *et al*.[7] and Wang *et al*.[28]. The acidogenicity of *S. mutans* is one of the most important indicators for dental caries, pH drop cause demineralization of tooth and development of the dental plaque. Due to the significantly inhibit acidogenicity of *S. mutans*, polyphenols from Pericarpium Granati were expected to become a good candidate for further development as an oral care agent for preventing dental caries.

CONCLUSION

In this study, HPD-450 was to select as the optimal resin of separation and purification of polyphenols from Pericarpium Granati and high-purity polyphenols was successfully separated and purified from the Pericarpium Granati extract according to the optimal parameters. The experiment of anti-*S. mutans* activity showed the purification of polyphenols exhibited significantly inhibited activity of growth, adherence and acidogenicity. It illustrated that polyphenols is the one of anticaries material basis of Pericarpium Granati extract. Therefore, further studies are worth to perform and it is expected to become a good candidate for further development as an oral care agent for preventing dental caries.

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REFERENCES

1. Loesche, W.J., 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev., 50: 353-380.
2. Hasan, S., K. Singh, M. Danisuddin, P.K. Verma and A.U. Khan, 2014. Inhibition of major virulence pathways of *Streptococcus mutans* by quercitrin and deoxynojirimycin: A synergistic approach of infection control. PLoS One, Vol. 9, No. 3. 10.1371/journal.pone.0091736
3. Patel, K., A.K. Ali, N. Nair and V. Kothari, 2015. *In vitro* antibacterial activity of *Manilkara hexandra* seed extracts and violacein against multidrug resistant *Streptococcus mutans*. J. Nat. Remedies, 15: 1-11.
4. Smolarek, P.D.C., L.A. Esmerino, A.C. Chibinski, M.C. Bortoluzzi, E.B. dos Santos and V.A. Kozlowski, 2015. *In vitro* antimicrobial evaluation of toothpastes with natural compounds. Eur. J. Dentistry, 9: 580-586.
5. Veloz, J.J., N. Saavedra, M. Alvear, T. Zambrano, L. Barrientos and L.A. Salazar, 2016. Polyphenol-rich extract from propolis reduces the expression and activity of *Streptococcus mutans* glucosyltransferases at subinhibitory concentrations. BioMed Res. Int. 10.1155/2016/4302706
6. Jin, S.H., B. Ma, J.G. Zhou, Z.Q. Li and G.J. Bao, 2014. The research progress on Chinese herbs in preventing dental caries. Chin. J. Microecol., 26: 234-238.
7. Jiang, X.D., W.H. Yang, C.J. Zhou, K.L. Lu, Q.Y. Qin and C.W. Lin, 2016. Effect of *Bauhinia championii* (Benth.) Benth extract on *Streptococcus mutants in vitro*. Biomed. Res., 27: 758-761.

8. Li, H.X., Z. Wang and Y.Z. Liu, 2002. Progress in studies on chemical constituents and pharmacological effects of Punicaceae. Chin. Tradit. Herbal Drugs, 33: 765-769.
9. Zhang, Q., D.Y. Jia, K. Yao and Q. He, 2007. Purification of polyphenols from pomegranate peel by macroporous adsorbent resin. Fine Chem., 24: 345-349.
10. Zhu, J., J.J. Lu and Q.P. Yuan, 2010. Separation and purification of polyphenols from pomegranate husk by macroporous adsorbent resin. Food Sci. Technol., 35: 188-193.
11. Tang, L.L., L.W. Liu, L.F. Sun and X. Li, 2011. Study on purification of *Pomegranate peel* polyphenol by adsorbent resin. Food Res. Dev., 32: 48-52.
12. Tang, P.C., S.R. Jiao and Y.M. Tang, 2011. Advance of the extraction of *Pomegranate peel* polyphenol and its activity. J. Xihua Univ. (Nat. Sci. Edn.), 30: 98-102.
13. Ma, Q.Q., J.K. Li, Z. Yu, W. Zhao, R.R. Huang and Y.L. Zhang, 2013. Research progress in biological activities of *Pomegranate peel* polyphenol. Sci. Technol. Food Ind., 34: 363-366.
14. Wang, B.S., K.L. Leu, G.J. Huang, C.F. Yeh, H.M. Tai, W.Y. Ho and M.H. Huang, 2014. Protective effects of an aqueous *Pericarpium granati* extract against inflammatory damage in mice. J. Funct. Foods, 9: 183-191.
15. Dorman, H.J.D., A. Peltoketo, R. Hiltunen and M.J. Tikkanen, 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected *Lamiaceae* herbs. Food Chem., 83: 255-262.
16. Jiang, X., Y. Liao, G. Lu and Z. Xiao, 2015. Response surface optimized ultrasonic assisted extraction of total flavonoids from QingLi Cao and *in vitro* antioxidant activities. Open Biotechnol. J., 9: 134-142.
17. Wang, R., X. Peng, L. Wang, B. Tan, J. Liu, Y. Feng and S. Yang, 2012. Preparative purification of peoniflorin and albiflorin from peony rhizome using macroporous resin and medium-pressure liquid chromatography. J. Sep. Sci., 35: 1985-1992.
18. Deng, T., J.F. Jia, N. Luo and H. Li, 2014. A dual-task method for the simultaneous detoxification and enrichment of stilbene glycoside from *Polygonum multiflorum* roots extract by macroporous resin. Chem. Eng. J., 237: 138-145.
19. Buran, T.J., A.K. Sandhu, Z. Li, C.R. Rock, W.W. Yang and L.W. Gu, 2014. Adsorption/desorption characteristics and separation of anthocyanins and polyphenols from blueberries using macroporous adsorbent resins. J. Food Eng., 128: 167-173.
20. Li, C., Y. Zheng, X. Wang, S. Feng and D. Di, 2011. Simultaneous separation and purification of flavonoids and oleuropein from *Olea europaea* L. (olive) leaves using macroporous resin. J. Sci. Food Agric., 91: 2826-2834.
21. Wen, Y., Y.T. Liu, J.M. Zhang and L. Guo, 2013. Purification of total flavonoids from *Buddleja officinalis* by AB-8 macroporous adsorption resin. Adv. Mater. Res., 641-642: 988-992.
22. Liu, Y., J. Liu, X. Chen, Y. Liu and D. Di, 2010. Preparative separation and purification of lycopene from tomato skins extracts by macroporous adsorption resins. Food Chem., 123: 1027-1034.
23. Khurram, M., A. Hameed, M.A. Khan, M.U. Amin and M. Hassan *et al*., 2012. Antibacterial potentials of *Quercus baloot* Griff. J. Med. Plants Res., 6: 1244-1249.
24. Limsong, J., E. Benjavongkulchai and J. Kuvatanasuchati, 2004. Inhibitory effect of some herbal extracts on adherence of *Streptococcus mutans*. J. Ethnopharmacol., 92: 281-289.
25. Rahim, Z.H.A. and H.B.S.G. Khan, 2006. Comparative studies on the effect of Crude Aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of *Streptococcus mutans*. J. Oral Sci., 48: 117-123.
26. Joycharat, N., S. Thammavong, S. Limsuwan, S. Homlaead and S.P. Voravuthikunchai *et al*., 2013. Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*. Arch. Pharm. Res., 36: 723-730.
27. He, K., L. Li, Y. Wu and D.R. Su, 2001. The study of extracts *Ginkgo biloba* Lon anti-adhesion of *Streptococcus mutans*. J. Guangxi Med. Univ., 18: 323-324.
28. Wang, Y., W. Li, Y. Yin, Z. Cao, L. Ming and W. Li, 2009. The protective effects of Shexiang Xingnaoning on global cerebral ischemia reperfusion injury. Acta Universitatis Medicinalis Anhui, 44: 475-479.